Purification of Recombinant Xylosyltransferase XXT2 Involved in Xyloglucan Biosynthesis

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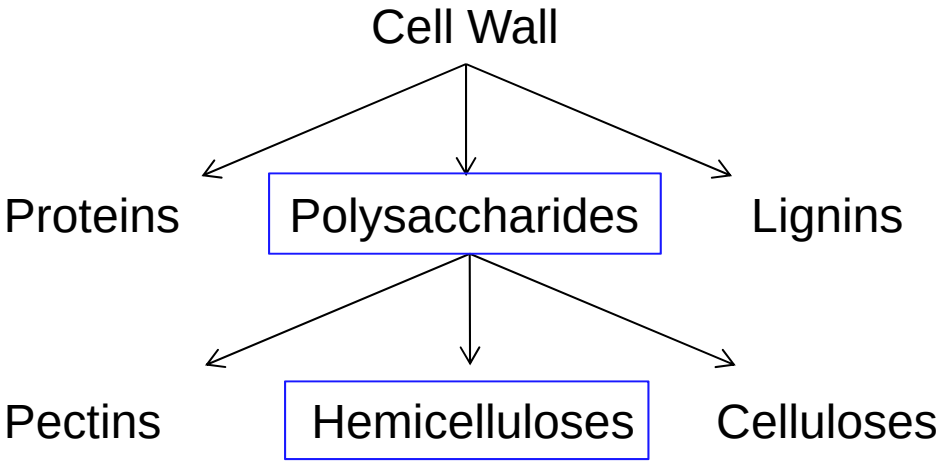
Plant Cell Walls

- Food, Fuel, Fiber

Soybean Plant, Botanical Garden, Karlsruhe, Germany, August 2009
Credit: H. Zell, GNU Documentation License



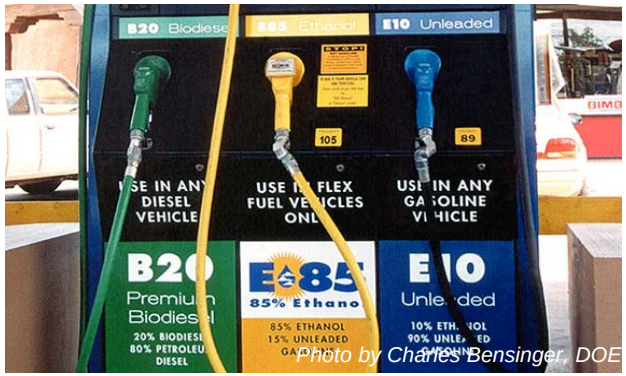
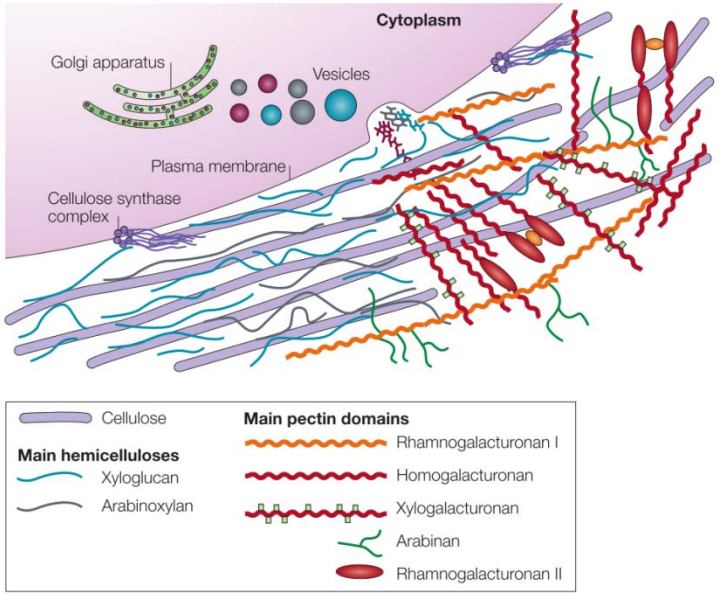


Photo by Charles Bensinger, DOE

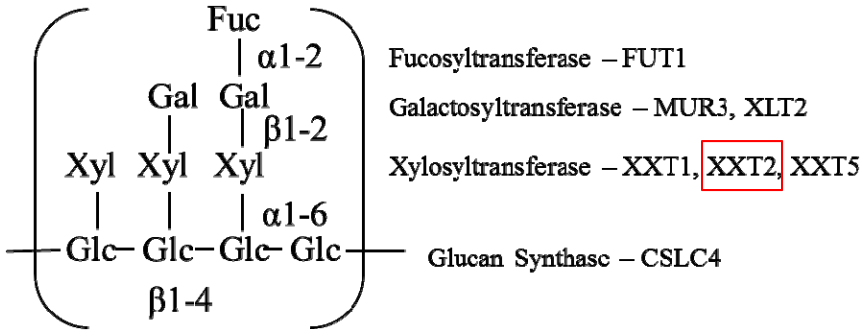
Greendragongroupltd.com

Xyloglucan

- Hemicellulose – branched polymer
- Primary Cell Wall



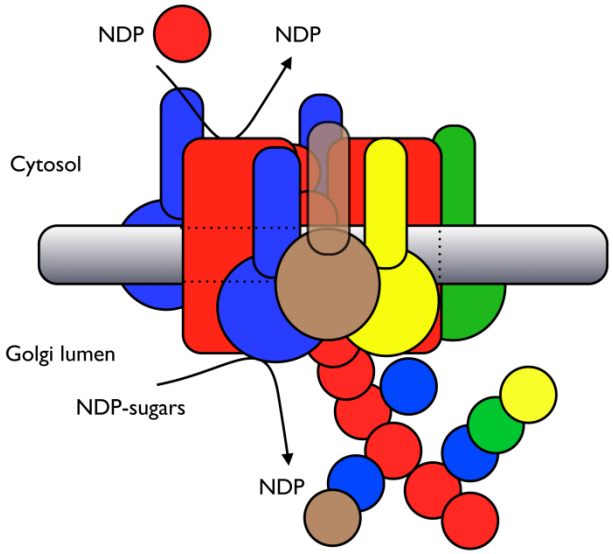
Cosgrove et. al 2005



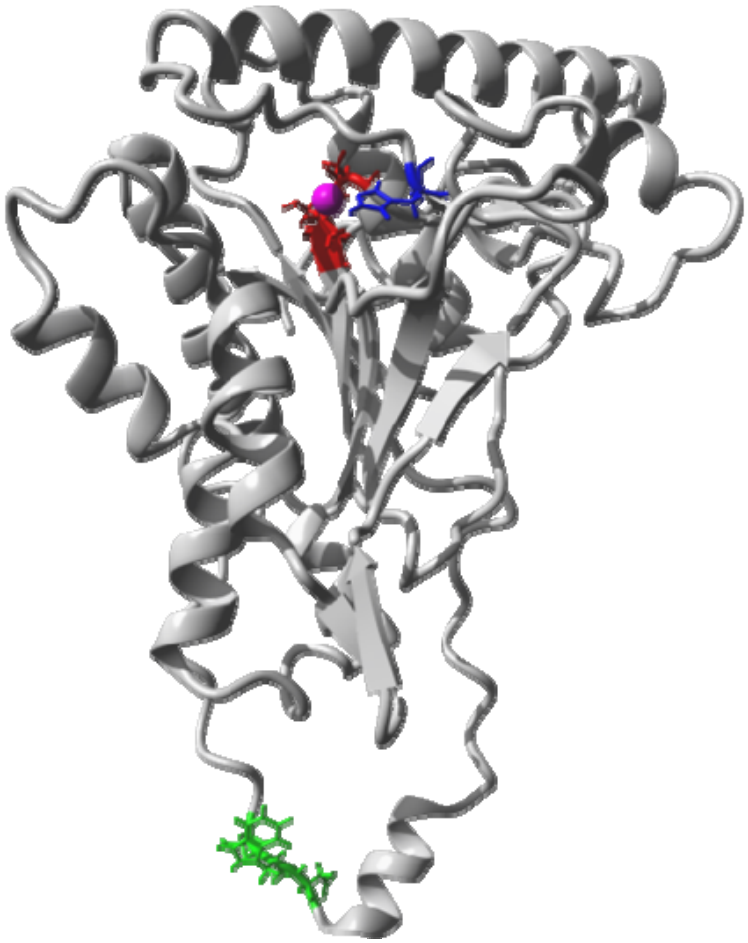
Xyloglucan subunit

Xyloglucan Biosynthesis

- Multiprotein Complex
- Homo/Hetero-Complexes



XXT2 Computational Model



XXT2

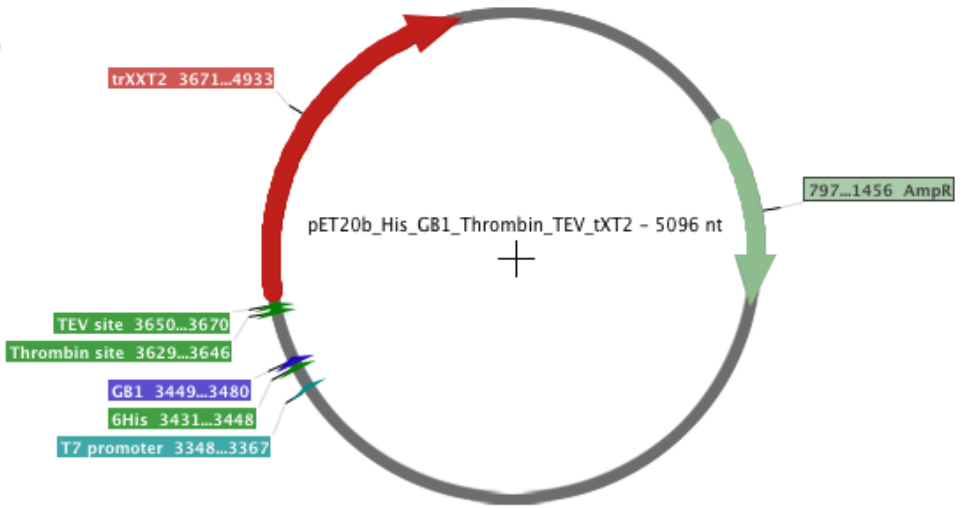
Dr. Alesia Tietze
YASARA

Goals

- Optimize Protocol
- Accumulate Protein
- Biophysical Studies
  - ITC
  - Mutagenesis
  - Crystallography

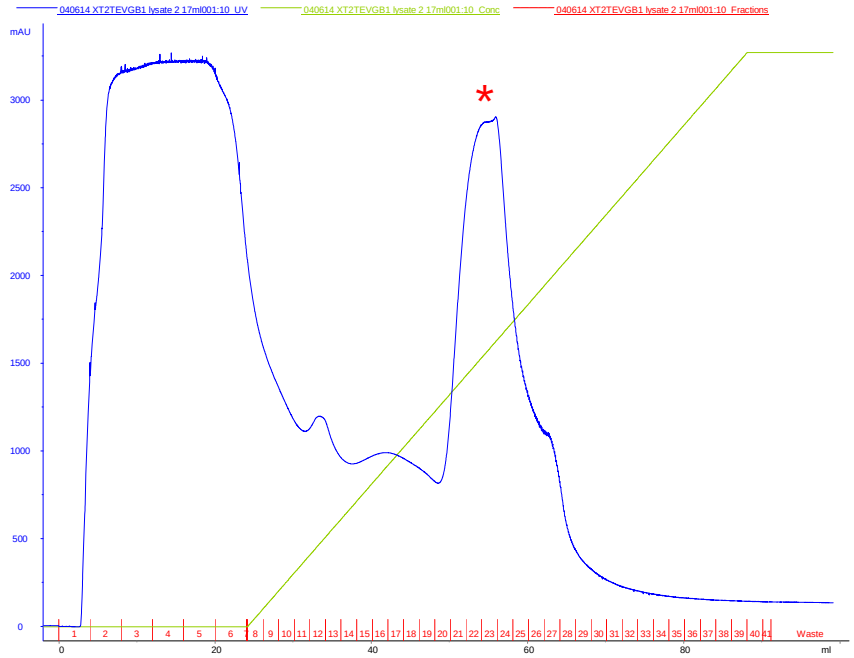
Bacterial Protein Expression

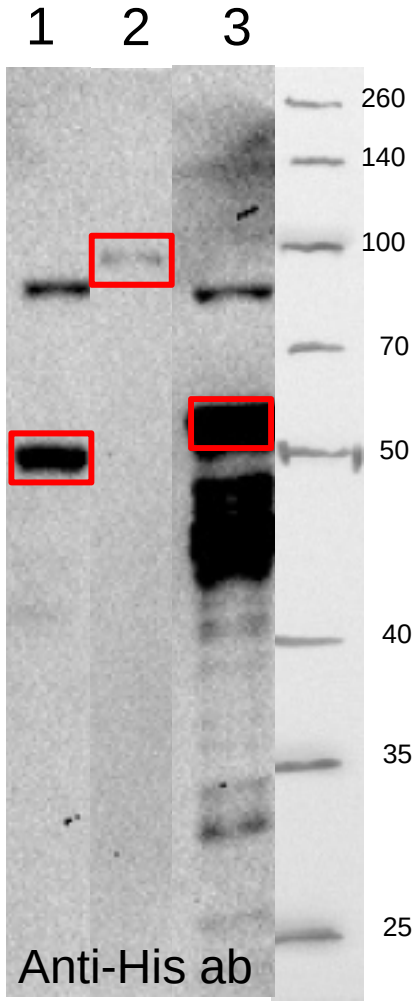
- Truncated XXT2
- Cleavage Site
- Fusion Tag (GB1, MBP, GFP)
- *E. Coli* (SoluBL21)



Protein Purification

- Affinity Chromatography (Ni-NTA/6xHis)

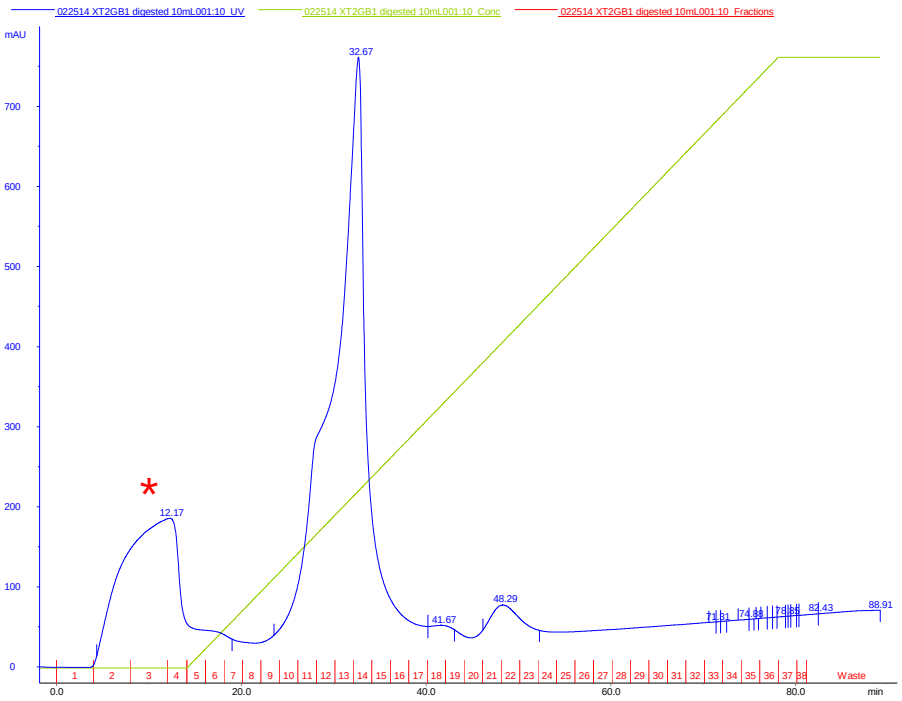


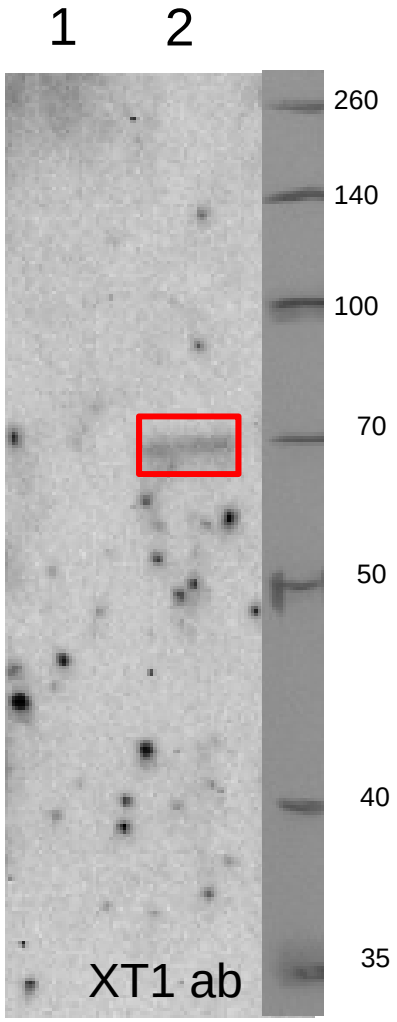


1) XXT2-His = 49 kDa
2) XXT2-MBP = 90 kDa
3) XXT2-GB1 = 56 kDa

Protein Purification

- Protease Cleavage

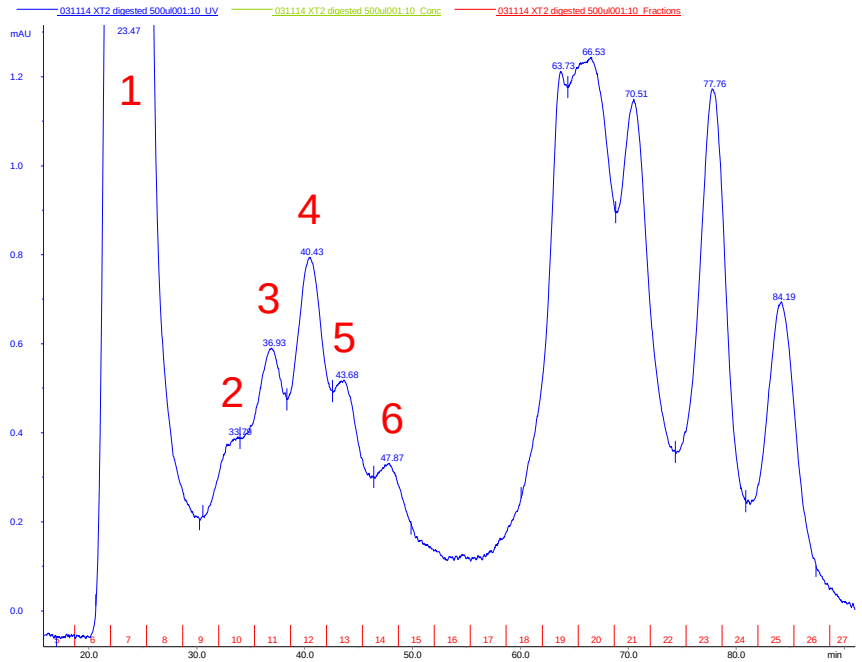


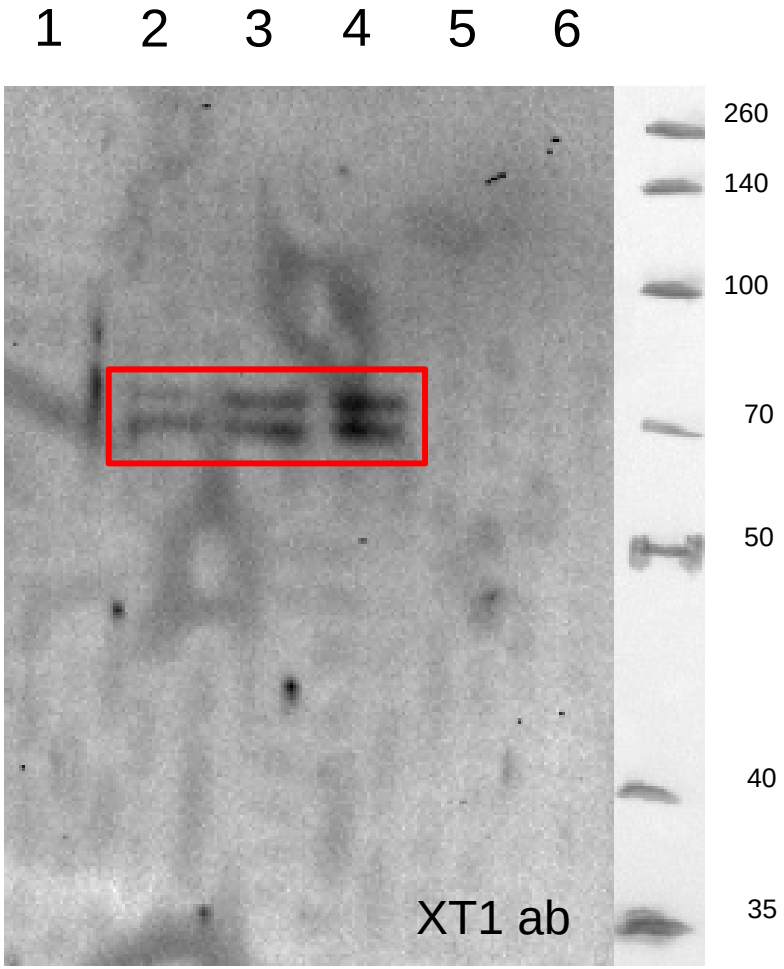


1) XXT2(-MBP)
2) XXT2(-GB1)

Protein Purification

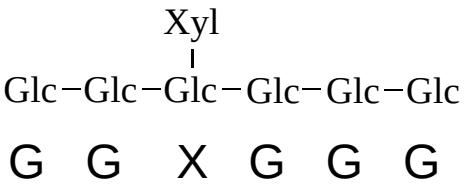
- Gel Filtration

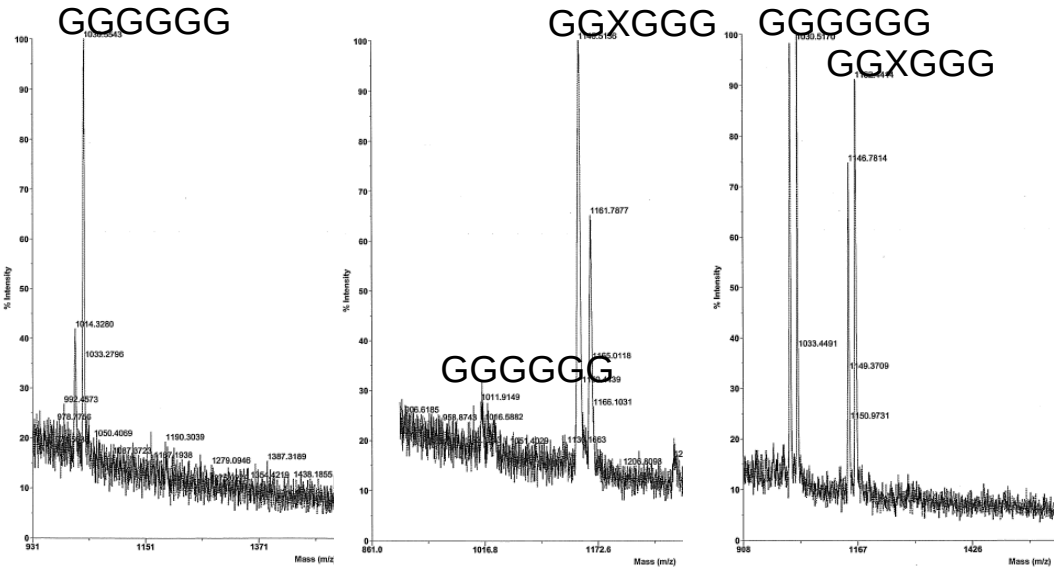




XXT Activity Assay

- Cellohexaose
- UDP-Xylose
- MALDI-TOF MS





Negative Control | Positive Control (XXT1-GB1) | XXT2-MBP

Fraction	Mass (Da)
GGGGGG + Na^+	1013.7
GGXGGG + Na^+	1145.8

Conclusions and Future Work

- Developed protocol for expression and purification
- Expression of active protein confirmed
- XXT2-MBP has activity, but low concentration
- Continue with XXT2-GB1 (higher concentration)
- Biophysical studies

Acknowledgements

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Questions?